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SERUM ANTIBODIES REACTANT WITH KOREAN HAEMORRHAGIC FEVER AGENT --ETC(U)
JUN 80 G FRIMAN, G R FRENCH, L HAMBRAEUS

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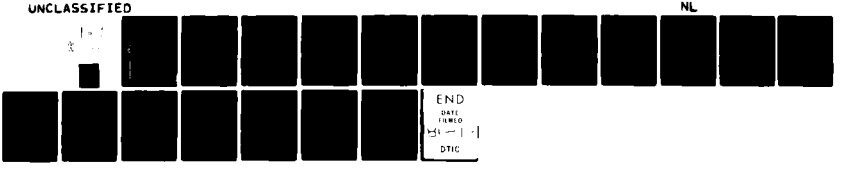

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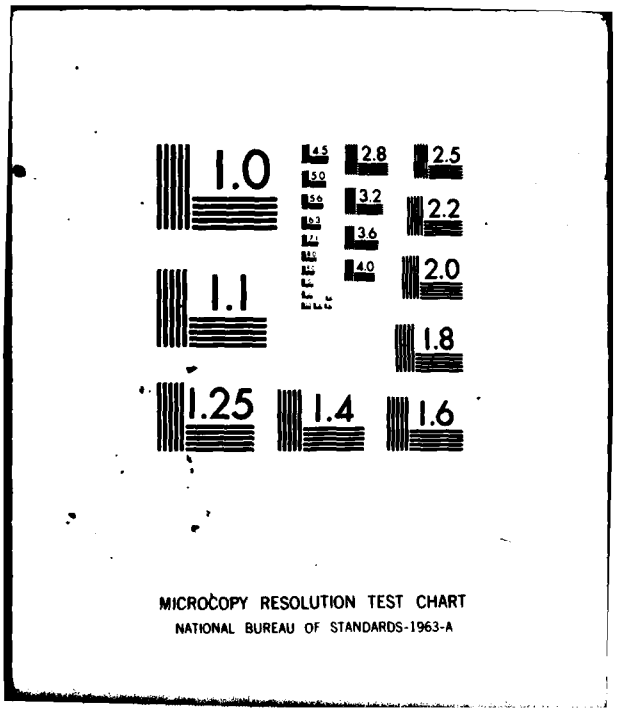
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A newly developed spot slide immunofluorescence method utilizing an in vitro antigen source was used for the first time for the assay of antibodies reactant with the Korean haemorrhagic fever (KHF) agent in sera from patients diagnosed with Scandinavian endemic benign (epidemic) nephropathy (nephropathia epidemica, EBN) and from 42 age-matched control patients living in the same area but suffering from other maladies. KHF antibodies were demonstrated in all of 14 EBN patients who were followed prospectively, 7 of whom exhibited seroconversion, and in 6 of 8 EBN patients studied retrospectively, but (cont'd)

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ABSTRACT

A newly developed spot slide immunofluorescence method utilizing an in-vitro antigen source was used for the first time for the assay of antibodies reactant with the Korean haemorrhagic fever (KHF) agent in sera from patients diagnosed with Scandinavian endemic benign (epidemic) nephropathy (nephropathia epidemica, EBN) and from 42 age-matched control patients living in the same area but suffering from other maladies. KHF antibodies were demonstrated in all of 14 EBN patients who were followed prospectively, 7 of whom exhibited seroconversion, and in 6 of 8 EBN patients studied retrospectively, but in only one of the 42 controls. Similar to that seen in KHF, antibodies in EBN appeared within the first week of onset of symptoms and persisted for long periods of time. The time from the onset of the illness until maximal antibody titre was recorded varied from 9 days to 1 month. On average, the level of the antibody titres measured to EBN was lower than that usually encountered in the Korean disease. The results indicate a close antigenic relationship beteen the KHF and EBN agents and demonstrate that the reliability of our new spot slide method is similar to that of another previously reported and more laborious immunofluorescence method using lung sections from infected rodents as antigen source.

INTRODUCTION

The aetiological agent of Korean haemorrhagic fever (KHF) has recently been isolated from the tissues of naturally infected field mice of the species Apodemus agrarius Coreae (Lee et al. 1978a). Antibodies to this agent, which is apparently a lipid solvent sensitive virus (Lee et al. 1978a, b), have been demonstrated in sera of KHF patients, using an indirect immunofluorescence method in which lung sections from infected rodents are used as an antigen source (Lee et al. 1978a). A haemorrhagic nephrosonephritis clinically similar to KHF is endemic in Russia (Gajdusek 1953) and antibodies to the KHF agent have also been found in sera from patients with this disease (Lee et al. 1978a).

Endemic benign (epidemic) nephropathy (EBN) or nephropathia epidemica Scandinavica, described for the first time in the 1930s (Myhrman 1934, Setterholm 1934), shows several clinical and epidemiological similarities to KHF but generally it is milder and has little or no lethality (Lahdevirta 1971, Nystrom 1977). Lee et al. (1979) last year reported findings of antibodies to the KHF virus in sera from 20 Finnish EBN-patients and Svedmyr et al. (1979) reported similar findings in sera from 18 out of 26 Swedish patients with suspected EBN. In both studies the indirect immunofluorescent technique was used, employing frozen acetone-fixed lung sections from infected A. agrarius Coreae (lee et al. 1978). Very recently Brummer-Korvenkontio et al. (1980), using the method of

Lee et al. (1978), reported the successful detection of an antigen in lung sections of bank voles (Clethrionomys glareolus) in Finland that reacted with antibodies in sera of 16 EBN patients.

Recently one of us (G.R.F.) was successful in propagating the KHF virus in cultured A-549 human lung carcinoma cells (French et al. 1980) and a infected cultured cell-spot slide immunofluorescence method was developed for assay of antibodies to the KHF agent. This method was employed in the present controlled study of occurrence of antibodies to the KHF virus in sera of patients who fell ill in 1976-79 with symptoms and signs compatible with a diagnosis of EBN. All patients lived in the county of Vasterbotten in northern Sweden where EBN is known to be endemic. This study has one prospective and one retrospective part. Friman et al. (1980) reported preliminary results of initial prospective findings. The purpose of the additional study was to obtain sera for the retrospective study of antibody titres in the early course of the disease and 1-2 years thereafter.

MATERIAL AND METHODS

Patients

Patients admitted to the department of Infectious Diseases, Umea University Hospital took part in the study. The clinical criteria used for discriminating cases of EBN were as follows (Nystrom 1977): 1. Sudden onset of illness, 2. Elevated body temperature, 3. Ache and/or pain, mostly abdominal or in the back, 4. Gastrointestinal symptoms, 5. Proteinuria,

6. Elevated serum nonprotein nitrogen concentration, 7. Uneventful course, and 8. Spontaneous recovery. In addition, the urinary sediments were investigated in some of the patients, since this has recently been reported in EBN and often show findings that are virtually pathognomonic for this disease (Wahlin et al. 1977). Histories did not reveal EBN-like diseases on earlier or later occasions in any of the patients including the controls in the study.

Retrospective group. For the retrospective part of the study, sera were used from 8 patients who had been hospitalized with EBN in 1976-77. In all but 3 patients of the retrospective group, criterium No. 3 or 4 was missing. In 2 out of 3 patients studied, a typical urinary sediment was demonstrated, and all displayed a transitory elevation of serum creatinine concentration (maximal value 381.6 $\pm$ 82.0 µmol/l). All but 2 had oliguria followed by polyuria and in 4, an exposure to small rodents was recorded. Follow-up sera were obtained 1 to 2 years after the onset of illness.

Control group. Sera from 42 patients admitted to the department in 1976-79 with other maladies than EBN were used as controls. The age, sex distribution and area of domicile living were similar to the EBN groups.

Data on the patients are included in Table I. For various practical reasons no additional sera from the present patients were available for analysis other than those included in the results below.

Serologic Assay for KHF Antibody: Antibody titers were determined by the indirect fluorescent antibody technique utilizing A-549 line human lung carcinoma cells (American Type Culture Collection, Rockville, Maryland; CCL 185) infected with the 9th A-549 passage level of KHF virus derived from the ~~3rd~~ 5th Apodemus lung passage of strain 76-118 (Lee et al. 1978a). The cells were grown on teflon coated 6 mm diameter spot slides (Cell Line Associates, Minotola, N.J. Cat. #10-225-6), 10 spots/slide. Infected cells were obtained by inoculating drained, fully sheeted monolayers of A-549 cells in closed plastic flasks with the virus at an input multiplicity of 0.1 to 1.0 infectious virus/cell. Maintenance medium of 2% fetal calf serum in E-199 (Medium 199 in Earles Salts, Microbiological Associates, Rockville, Md.) was replaced and the inoculated cells were incubated 4 days at 36°C. At the end of the primary incubation period the infected cells were removed from the flask(s) by trypsinization, washed once in maintenance media and resuspended in growth medium of 10% fetal calf serum in E-199 to 1 x 10^6 cells/ml. Infected cells were then mixed with an equal volume and concentration of normal (not-infected) A-549 cells to provide a negative background and seeded onto pre-cleansed 10-spot slides sterilized with 70% ETOH and ultra-violet light. Five-one hundredths ml of cell suspension was dropped on each spot on slides arranged in stainless steel trays. The trays were then covered with aluminum foil and incubated an additional 24 hrs at 36°C in a 5% humidified CO_2 atmosphere. At 24 hours the fully sheeted spot slides were fixed in 100% cold acetone for 7-10 min at room temperature, air dried and stored at -70°C until used.

Test serum was titrated in two fold dilutions in phosphate buffered saline (PBS) pH 7.4. Generally, eight dilutions (1:20 to 1:1280) of one serum was tested on one slide. The remaining two wells on the slide were utilized for positive control serum (8 units of antibody at 1:80) and negative control serum (1:20). A conventional indirect fluorescent antibody test similar to that described by Lee and his associates (Lee et al. 1978a) utilizing 8 units of fluoroscein isothiocyanate conjugated goat anti human globulin antiserum (various commercial sources) was employed. Slides were read with a Leitz dialux 20 microscope equipped with a 50 watt mercury lamp in the incident light mode, 10X wide field occulars and a 15X objective. The endpoint was read as the highest dilution of serum that provided a clearly positive distinctive pinpoint pattern with the same proportion of infected cells (25-50%) observed with the control positive serum.

RESULTS

Results are included in Fig. 1.

Prospective group (patients Nos. 1-14 in Fig. 1).

Serum antibodies to the KHF virus could be demonstrated in all the patients, 7 of whom exhibited seroconversion. In 5 patients (Nos. 6, 7 and 11-13) a four-fold or higher titre rise from acute to convalescent-phase serum was recorded and in 2 patients (Nos. 2 and 3) a similar drop in antibody titre was observed during 8 months following the illness.

In only 1 patient did the antibodies disappear from the serum during the observation period. In that single patient (No. 14) antibodies were barely detectable in the 3.3 months sample and were absent from the 10.6 month sample.

Antibodies were present in acute phase sera in all but 2 patients (Nos. 11 and 13). In those 2 patients as in a third patient (No. 12) who showed a low acute phase antibody titre, a four-fold antibody rise was delayed to 1 month or more after the onset of the symptoms.

Retrospective group (patients Nos. 15-21 in Fig. 1).

In all but 2 of these patients, KHF antibodies were present in acute phase or later serum samples, or both. In one patient (No. 20) both an early and a late serum were negative. Coxsackie virus, type B4, was isolated from the stools of this patient in the acute phase. In the other sero-negative patient (No. 17) the serum sample was drawn on the fourth day of the illness and no later serum was available. In a third patient (No. 19) an acute phase serum was negative, although it had been drawn as late as the seventh day of symptoms. This patient later become sero-positive.

A second serum sample was obtained 1.5 - 1.8 years after the acute disease from 5 of the 8 patients studies in the retrospective group. Four patients (Nos. 18, 19, 21 and 22) had detectable KHF antibody in these late sera.

Control group. In sera from all but one of 42 control patients, KHF antibody could not be demonstrated. In this one patient, as in the other control patients, a negative history of SEN or SEN-like illnesses was obtained.

DISCUSSION

In the present study, for the first time, an immunofluorescence method using infected cultured A-549 line human lung carcinoma cells has been used in a study of antibody in EBN. The method was made possible by the recent development at the USAMRIID of the capability to propagate the KHF agent in an in vitro cell line (French et al. 1980). It has proved reliable and reproducible for the detection of KHF antibodies.

In the present prospective study, antibodies reactant with the KHF agent have been found in all of 14 EBN patients. In order to study early and late antibody events further, a retrospective group of 8 EBN patients was added. The absence of antibodies in sera from 2 of these patients may be explained by a misdiagnosis and infection with Coxsackie virus, type B4, in one of the cases and lack of follow-up serum samples in the other.

The fact that seroconversion was recorded in several patients, and that all but one of 42 age-matched control patients with no previous history of EBN, and living in the same endemic area as the EBN patients were seronegative, in combination with the finding of antibodies in serum persisting for years after the acute disease, support the view that the

clinically defined disease of EBN (Lahdevirta 1971, Nystrom 1977) is caused by an agent related to the KHF virus. A similar prolonged persistence of antibodies in sera of EBN patients was recently reported by Brummer-Korvenkontio et al. (1980) using an EBN agent antigen that had been isolated from bank voles (Clethrionomys glareolus) in Finland.

The antibody titres were found in these two studies on the average lower than those that have been measured in KHF patients (Lee et al. 1978, French et al. 1980) suggesting that antigenic differences may exist between the EBN and KHF agents. Similar antibody titres have recently been reported in EBN patients by Lee et al. (1979) and by Svedmyr et al. (1979) using KHF infected Apodemus agrarius lung sections as antigen source (Lee et al. 1978). The findings of only slightly higher antibody titres when using the Finnish bank vole source antigen (Brummer-Korvenkontio et al. 1980) are therefore somewhat unexpected but may be explained by possible differences in conjugation and fluorescent microscopic technologies.

In KHF, antibodies become detectable in most cases in serum drawn 7 days after the onset of symptoms (Lee et al. 1978). In the present study 7 out of 12 EBN patients displayed antibodies within the first week of the illness, and all were positive after the first week. In fact, one patient was sero-positive as early as the third day of the illness. A similar early appearance of antibodies in EBN has been reported by Lee et al. (1979) and Brummer-Korvenkontio et al. (1980).

The time from appearance of symptoms until maximal titres of antibodies were recorded varied substantially between patients. On one extreme, 5 patients reached their recorded maximum as early as on their 9-12th day of illness. Conversely, in 3 patients, antibody titres rose conspicuously slower and seroconversion occurred as late as 1 month or more after onset. Brummer-Korvenkontio et al. (1980) observed similar slow rises in antibody titre in 3 of their patients using the Finnish antigen. It is open to speculation whether this variable timing in antibody response is explained by differences in host response factors or in the nature of the resulting illness. The long-standing antibody titres following EBN and the lack of antibodies in 41 of 42 controls from the same geographic area indicate that subclinical EBN with humoral immune response is probably not very common.

Legend to Table and Figure

Table I. Age, sex distribution and Nos. of sera.

Fig. I. Immunofluorescent serum antibody titres (reciprocal serum dilutions) in EBN in relation to onset of symptoms. Patient numbers are shown on the ordinant and time intervals along the abscissa.

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Goran: I doubt that you'll get away with this. I suggest instead until they actually Publish it.

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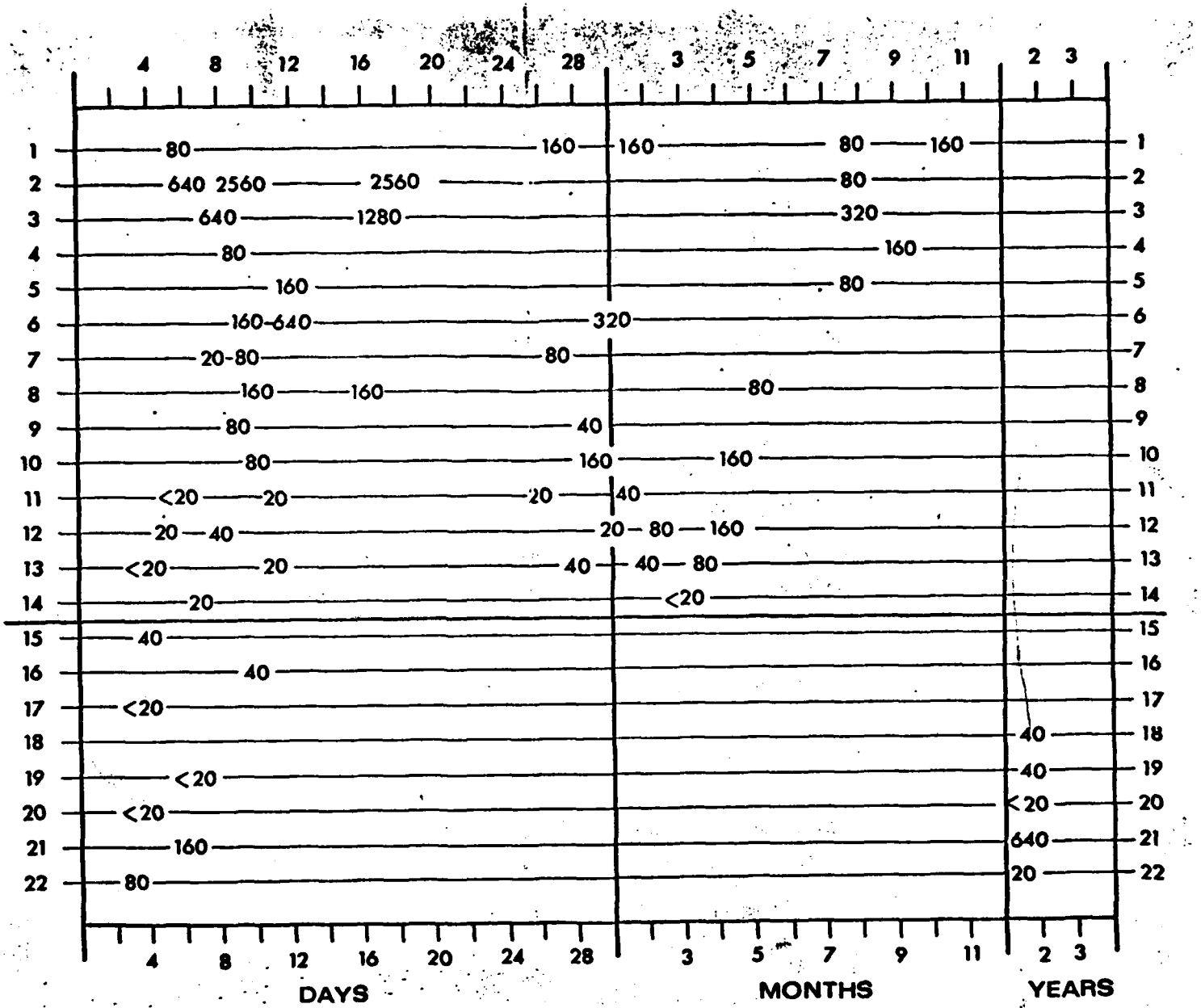
Table I

	Prospective group	Retrospective group	Control group
No. of patients --			
Males	10	7	24
Females	4	1	18
Total	14	8	42
Mean age (range)	47.2 (30 - 65)	37.6 (9 - 63)	52.9 (25 - - 65)
No. of sera	46	12	42


PROSPECTIVE GROUP
RETROSPECTIVE GROUP
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3 5 7 9 11
2 3
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2 640 2560 2560 80
3 640 1280 320
4 80 160
5 160 80
6 160-640 320
7 20-80 80
8 160 160 80
9 80 40
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